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Isolation of Western Equine Encephalomyelitis Virus from Tropical Fowl Mites, *Liponyssus bursa* (Berlese).*

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At the present time the exact mode of transmission of western equine encephalomyelitis is far from established, and the mechanism by which the causative agent persists from year to year in a given area has not been fully explained. Numerous attempts have been made to determine reservoir hosts and vectors of the disease. Hammon and his associates[1] demonstrated that a large per cent of both domestic and wild birds possess neutralizing antibodies for this virus suggesting that the reservoir host might be one closely associated in some manner with domestic fowl. It seemed likely, as pointed out by Smith and her associates,[2] that some blood-sucking vector which does not necessarily bite man was transmitting the disease to fowl. These investigators recently reported the isolation of the St. Louis encephalitis virus from naturally infected chicken mites, *Dermanyssus gallinae* (DeGeer), in the St. Louis area where the disease is endemic. Later, they reported several additional isolations from chicken mites, and demonstrated transovarian transmission of the virus from naturally infected female mites.[3] Shortly thereafter, Sulkin[4] reported the isolation of the Western equine virus from naturally infected chicken mites in the Dallas area during the epidemic year of 1944. During the following non-epidemic year additional attempts to demonstrate this virus in chicken mites and fowl ticks collected in this identical locality and other areas in Texas, were unsuccessful. Numerous attempts by Hammon and his associates[5] to demonstrate virus in this ectoparasite have been negative. Since the possible role of the chicken mite in the transmission and effective perpetuation of the virus encephalitides is not yet clarified, it was considered desirable to continue the search for a reservoir among other avian blood-sucking ectoparasites.

Recently Reeves and his collaborators[6] reported recovery of the Western equine virus from wild bird mites, *Liponyssus sylviarum* (Canestrini and Fanzago), in Kern County, California. The present report concerns an additional isolation of the Western equine virus from similar mites collected in Dallas County, Texas from a nest containing two live nestling English sparrows, *Passer domesticus* (Linn.).† The nest was brought into the laboratory on July 9, 1947. A layer of white absorbent cotton was placed over the top, and the entire nest was then placed in a covered glass container. On the following day, mites identified as *Liponyssus bursa* (Berlese),† were collected from the cotton and the underside of the lid, and were tested

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1 Hammon, W. McD., Lundy, H. W., Gray, J. A., Evans, F. C., Bang, F., and Izumi, E. M., *J. Immunol.*, 1942, **44**, 75; Hammon, W. McD., Reeves, W. C., and Irons, J. V., *Texas Rep. Biol. and Med.*, 1944, **2**, 366.

2 Smith, M. G., Blattner, R. J., and Heys, F. M., *Science*, 1944, **100**, 362.

3 Smith, M. G., Blattner, R. J., and Heys, F. M., Proc. Soc. Exp. Biol. and Med., 1945 **59**, 136; *J. Exp. Med.*, 1946, **84**, 1.

4 Sulkin, S. E., *Science*, 1945, **101** 381.

5 Hammon, W. McD., and Reeves, W. C., *Am. J. Pub. Health*, 1945, **35**, 994.

6 Reeves, W. C., Hammon, W. McD., Furman, D. P., McClure, H. E., and Brookman, B., *Science*, 1947, **105**, 411.

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in 3 pools for the presence of a neurotropic virus. The three pools, each containing approximately 300 mites (adults and nymphs), were aspirated into small vials as described elsewhere[7] and allowed to remain overnight. Each pool was ground in a mortar with 2.0 cc of nutrient broth, and then centrifuged at 5000 r.p.m. for 15 minutes in an angle centrifuge. Approximately 0.3 cc of the supernatant fluid, which was cultured aerobically and anaerobically, was injected intraperitoneally into each of 5 Swiss mice. Pool No. 1 was inoculated into 8-day-old mice and pools No. 2 and No. 3 into 12-day-old mice. The supernatant fluids did not contain enough bacteria to affect the animals. An agent later identified as western equine encephalomyelitis virus was recovered from pool No. 1 and after 4 serial passages in Swiss mice, filtration experiments (Berkefeld N filter) were conducted with the pooled brains of mice showing neurological symptoms. Identification of a filterable agent of the sixth passage as the Western type of equine encephalomyelitis virus was made by neutralization tests with serum of rabbits immunized to known strains of Western and Eastern equine virus and St. Louis encephalitis virus. The virus recovered from the sparrow mites was neutralized by the Western equine immune serum but was not affected by the serum of rabbits immunized to the Eastern strain of equine virus or the virus of St. Louis encephalitis. With each passage, before and after the filtration experiment, aerobic and anaerobic cultures were made and brain tissue from animals showing central nervous system symptoms were examined for histopathological evidence of encephalitis. Microscopic sections of a mouse and a guinea pig brain from the second intracerebral passage showed histopathological evidence characteristic of encephalitis. Pools No. 2 and No. 3 of mites collected from the same source but injected into older mice yielded negative results. All attempts to isolate a filterable virus from 11 additional pools of specimens of wild bird mites yielded negative results. Additional attempts to demonstrate virus in chicken mites were likewise negative. Studies are still in progress to determine whether or not hereditary transmission of the Western equine virus in the chicken mite can be effected under experimental conditions. Similar studies will also be made with the wild bird mite.

Thus, in addition to the well documented evidence incriminating the mosquito in the transmission of western equine encephalomyelitis,[5,8] it is becoming increasingly apparent that other blood-sucking arthropods may be concerned in the transmission of the virus of equine encephalomyelitis.

Summary. The Western type of equine encephalomyelitis virus has been isolated from wild bird mites, *Liponyssus bursa* (Berlese), collected in the Dallas County, Texas area. At the present time the epidemiological role played by the wild bird mite and the chicken mite, *Dermanyssus gallinae* (DeGeer), from which the Western equine virus had been previously isolated, is unknown. The purpose of this report is to emphasize the importance of virus isolations from similar bird mites in two widely separated areas.

ADDENDUM: Two months after the Western equine virus was isolated from the tropical bird mites collected in Dallas County, the Eastern equine virus was recovered from a horse brain submitted to us from Jefferson County, Texas, where a widespread epizootic was occurring. This would indicate that both the Western and Eastern equine types of encephalomyelitis were active in Texas at about the same time.

7 Wisseman, C. L., Jr., and Sulkin, S. E., *Am. J. Trop. Med.*, 1947, **27**, 463.

8 Hammon, W. McD., Reeves, W. C., Brookman, B., and Izumi, E. M., *J. Inf. Dis.*, 1942, **70**, 263.